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Addressing the Role of EBV in Breast Cancer

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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) In this grant we proposed to generate an EBV infected mammary epithelial system that could be used to characterize the life cycle and oncogenic properties of EBV in this unique tissue. We have been utilizing primary human mammary epithelial cell lines generated in Myles Brown's laboratory. By Fluorescence Activated Cell Sorting (FACS), we determined that these cells are positive for the EBV receptor, CR2 and we have been carrying out infection studies to determine the infectability of these cells to EBV. To date we have found that despite the presence of the CR2 receptor, infectability is relatively low. This makes transient studies more difficult. We have therefore, begun generating long term cultures of infected IMEC cells through a variety of different means. We believe that we are making encouraging progress but we estimate that the completion of this project will require another year to complete. We have therefore obtained a 1 yr no cost extension to allow us to complete this work.				
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INTRODUCTION:

EBV is a human herpes virus that infects B-lymphocytes as well as epithelial cells (9, 16, 18). EBV is the causal agent of infectious mononucleosis and is associated with the development of both B-cell and epithelial cell malignancies including the endemic form of Burkitt's lymphoma, post-transplantation lympho-proliferative diseases, AIDS-associated lymphomas, Hodgkin's disease, undifferentiated nasopharyngeal carcinoma (8, 13). EBV has also been linked to some cases of gastric carcinoma (2, 7, 17), T-cell lymphoma (15), and lymphoepithelioma-like carcinoma in the salivary glands, lung, and thymus (4, 14, 19). Although the possible role of EBV in breast cancer has been controversial, more recent publications have provided a potentially interesting correlation between EBV and breast cancer (1, 3, 5, 6, 10-12). This led us to propose investigating this relationship further. We proposed to generate a tissue culture model in which the biology of EBV in mammary tissues could be studied as well as its possible role in the initiation and/or maintenance of breast cancers.

BODY:

Since we found that human immortalized mammary epithelial cells (IMECs) express the CR2 receptor, we believed that we could infect these cells with EBV to address EBV biology and EBV's influence on cell growth/transformation properties. Nevertheless, based on previous work in our lab as well as many other labs studying EBV, it is clear that EBV infection is typically low level despite the presence of CR2 on target cells. We have now addressed the efficiency of EBV infection in IMEC cells. For our initial studies, we generated virus from the B95-8 cell line after several days and high density. Using these conditions, we obtained <1% of cells infected with EBV as measured by immunofluorescence against EBNA1. In our application, we proposed to address the pattern of EBV gene expression during the initial stages of viral infection. Since these studies could be more readily carried out if we were able to obtain higher levels of infection, we carried out further experiments in an effort to obtain a higher infection efficiency. We tested whether concentration of virus generated from B95-8 cells yields higher infection efficiencies but infection efficiencies remained low. We therefore tested an alternative (although more expensive) method in an effort to obtain higher titers of EBV. Treatment of the EBV positive Burkitt's lymphoma cell line, Akata, with anti-Ig yielded significantly higher infection efficiency and we believe that these conditions will be sufficient to be able to carry out informative EBV gene expression studies.

We have also begun soft agar assays following infection to address whether EBV elicits any transformed phenotypes in IMECs. Although preliminary, our first set of experiments suggest that EBV indeed yields a substantially higher number of colonies in soft agar relative to control treated cells. If this result repeats, this is important not only from a conceptual standpoint but this will also be an effective means for us to select long term EBV expressing IMEC clones. This will allow us to address more of the EBV biology and gene expression patterns as well as cell cycle alterations that are conferred by EBV.

KEY RESEARCH ACCOMPLISHMENTS:

- Determined that IMEC cells express the EBV receptor, CR2.
- Identified infection conditions that are suitable for carrying out studies addressing the EBV gene expression profiles during initial infection.
- Obtained preliminary evidence that EBV enhances growth in soft agar.
- Begun the generation of long term IMEC cultures containing EBV.

REPORTABLE OUTCOMES:

- We are in the process expanding colonies from cultures infected with EBV. Pending characterization and cryopreservation, these cultures will be made available to other investigators.

CONCLUSIONS:

- 1) We have identified infection conditions that are suitable for characterizing the EBV gene expression program during initial infection. In addition, we have preliminary evidence that EBV enhances growth in soft agar. This will allow us to generate cell clones expressing EBV and leads investigations towards a possible role of EBV in the progression of breast cancer.

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